Boojum RESEARCH LIMITED

TITLE: STUDY OF THE TREATMENT OF ACIDIC SEEPAGES EMPLOYING A COMBINATION OF WETLAND ECOLOGY AND MICROBIOLOGY (50/50 COST SHARED)

FINAL REPORT

BY

Margarete Kalin
Boojum Research Limited

DSS FILE NO.: 039SQ.23440-8-9264

DSS CONTRACT SERIAL NO.: 23440-8-9264/01-SQ

CANMET PROJECT NO.: 2.31.1

CANMET SCIENTIFIC AUTHORITY: Mr. R.G.L. McCready

DATE: September 18, 1989

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21 DUNDAS SQUARE, SUITE 405, TORONTO, ONTARIO M5B 1B7, (416) 861-1086

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SECTION 1: Data Summary of the Foliar Fertilizer

UNIVERSITY OF WATERLOO

Initial Effects of Foliar Fertilizer on *Typha latifolia*

Work Report by:
Alan J. Unwin and Donald I. Stewart
Faculty of Environmental Studies
Faculty of Science
August 1989

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1.0 Executive Summary

The promotion of wetlands is essential in providing a biological solution to the problem of acid mine drainage. Cattails constitute a great portion of these wetlands and contribute a great deal to the accumulation of organic matter that is essential to the success of a biological polishing system. Biological polishing systems are designed to provide a self-sustaining and economical solution for treating acidic waters created by mining waste.

Establishing cattails in polishing ponds can be difficult due to the acidic conditions that prevail. Developing methods to achieve this goal is essential for cattail survival and growth. Fertilization is one such method that is being examined in this report. More precisely this report examines any possible initial detrimental effects that could occur after the application of a foliar fertilizer. Two types of fertilizers were applied to a natural and a transplanted stand of cattails using two different dilutions.

After examining select leaves from quadrats of natural and transplanted cattails it was found that minimal burning occurred in both types of fertilizers but was more evident in the 18-4-6 NPK type fertilizer. Tip burning was found on all leaves, including those from control quadrats, but could not be linked directly to the application of the fertilizer.

Overall it was recommended that fertilization continue under specified conditions so that solid conclusions could be made as to the effectiveness of the fertilizer.

2.0 Introduction

During mining activities treatment facilities are used to achieve an environmentally acceptable effluent. However, when the mine shuts down, these wastes represent an environmental liability for which no satisfactory, self-sustaining, economic treatment solution presently exists. When it was recognized that novel approaches to acid mine waste treatment problems were sparse, Boojum developed ecologically engineered reclamation schemes and biological polishing systems that could solve this problem.

Water quality is improved through the integration of precipitation processes in interceptor ditches and polishing ponds, followed by biological polishing processes which facilitate the adsorption of metals onto algal biomass which is relegated to the sediment (Kalin, 1988, 1987, 1986).

Mine tailings often contain large amounts of pyrite (FeS_2). The generation of sulfuric acid during oxidation of pyrite in tailings is as follows:

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \text{ --- } 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+$$

or $$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \text{ --- } 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+$$

This process is generally thought to be the result of microbial action under aerobic conditions. When acidification proceeds to less than pH 3, Fe3+ remains in solution and acts as an oxidizing agent.

Wetlands promote the accumulation of organic matter. This layer ultimately provides reducing conditions, and through the passage of water through sulfate reducing environments, microbial conversion of sulfate to H_2S occurs, resulting in metal precipitation. Organic matter also provides a catalytic surface for the precipitation of iron hydroxide. A schematic of wetland development and its ultimate self-maintaining state is outlined in figure 1 (Kalin and Van Everdingen, 1987).

Therefore, it is favorable to increase the growth rate of the cattails, as this will increase biomass and ultimately provide more polishing power. Wetlands also increase transpiration and thereby counteract infiltration (Larcher, 1980).

The purpose of this study is to develop a method of fertilization which will enhance the growth rate of existing cattails. This experiment will hopefully provide some insight as to the feasability of using a foliar fertilizer to achieve this goal.

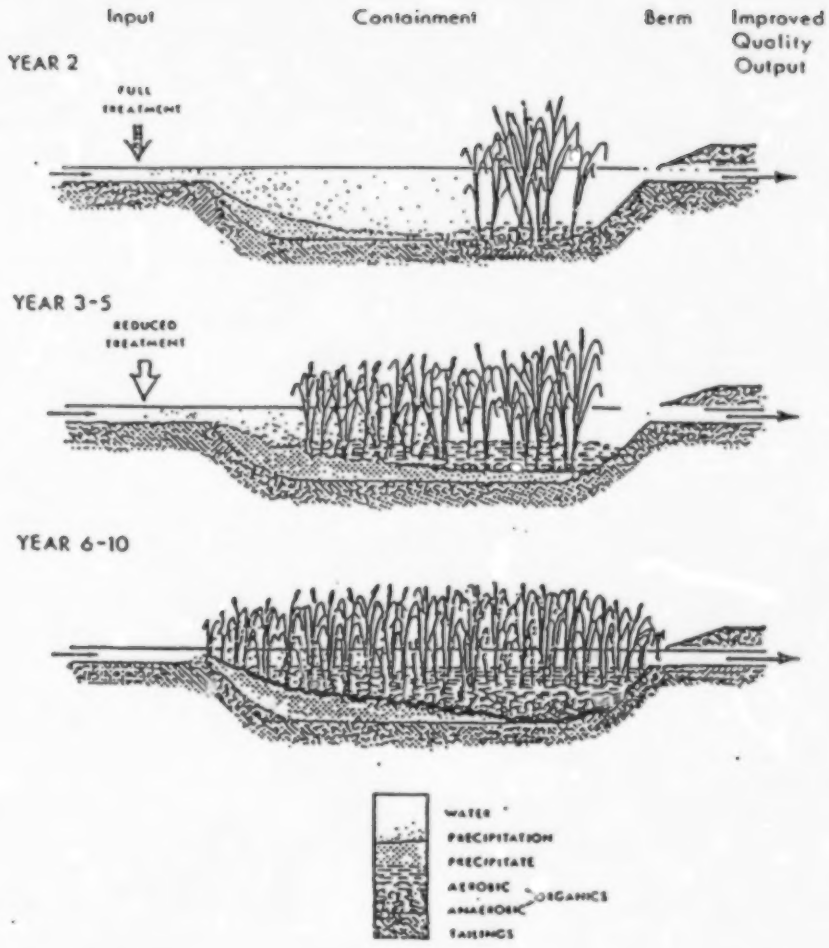


Figure 1: General Concept of Ecological Engineering

2.1 Objectives

Since only one application of the foliar fertilizer has taken place, surveying the cattails for any changes in growth or biomass would be unrealistic. A more pragmatic approach would be to observe any resulting effects from one such application of the fertilizer. Therefore, the main objective of this study is to examine any initial beneficial or detrimental effects that might result from the application of the foliar fertilizer.

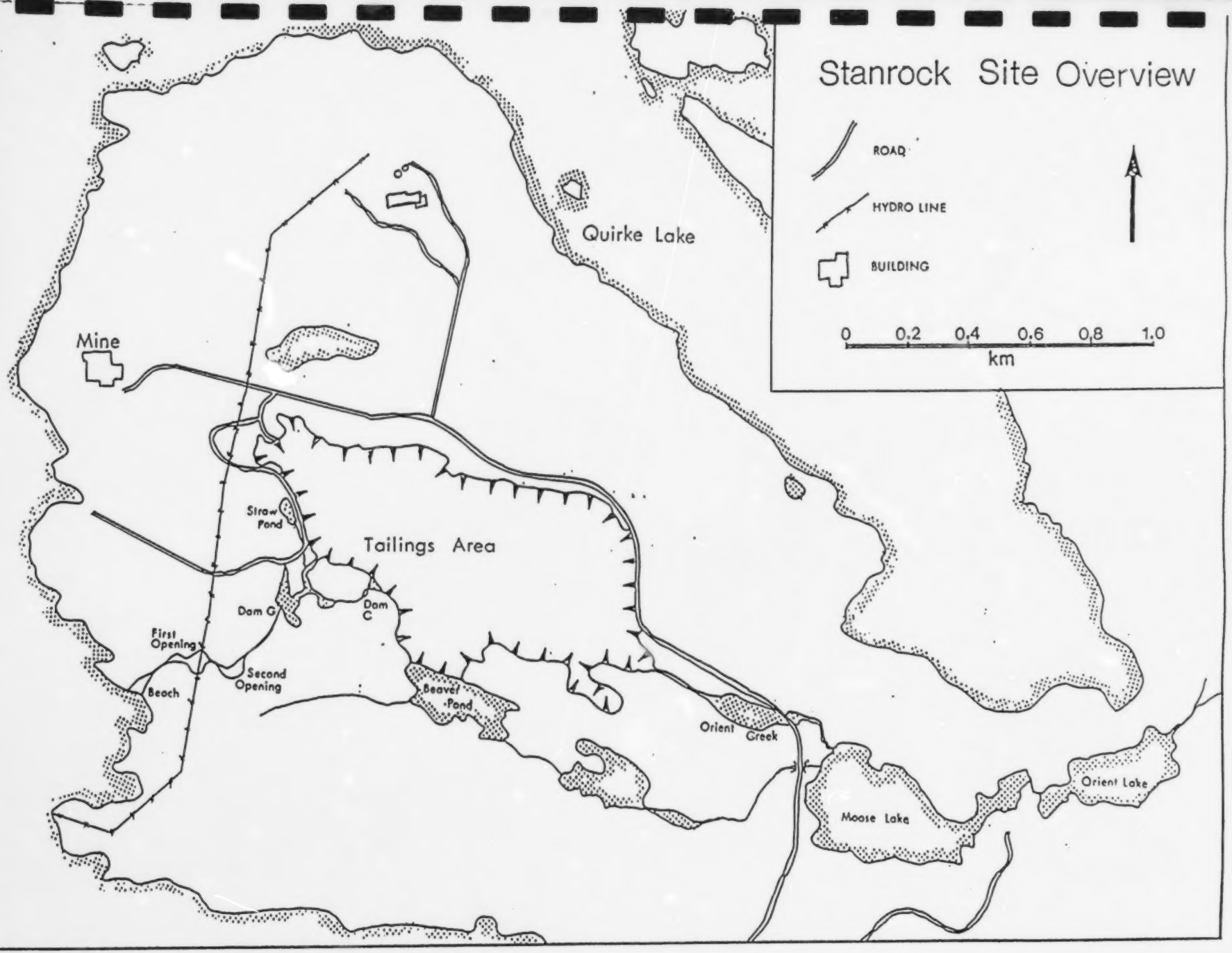
This will be accomplished by examining cattail leaves that have received and application of foliar fertilizer and comparing them with leaves from an untreated control area.

From these observations, conclusions and recommendations will be made on whether the application of foliar fertilizer should proceed, and under what conditions this continuation should take place.

2.2 Site Description

Acid mine drainage (AMD) water is currently collected in a seepage pond, Dam G (Refer to Map 1). This water is presently pumped back to a treatment plant where lime is added to neutralize the water. Below Dam G, a small seepage creek leads into the second opening (Refer to Map 1). The upper half of this area consists mainly of bare tailings. On the east side, some seepage flows from the direction of Beaver Pond (Refer to Map 1). At the south end of the second opening, a small cattail stand remains from a 1987 transplant.

The first opening, which is the experimentation site, is bordered by natural cattail stands. The seepage flows on the northwest perimeter of the opening and is diverted to the central area. This central area contains transplanted cattails from 1987 and 1988. The seepage continues on through a large stand of natural cattails where it ends at Quirk Lake.



Map 1: Stanrock Site Overview

3.0 Methodology

Two different types of *Stoller Harvest Plus foliar* fertilizer were used both with different N-P-K. One with a N-P-K of 14-4-6 and the other with a 4-18-6.

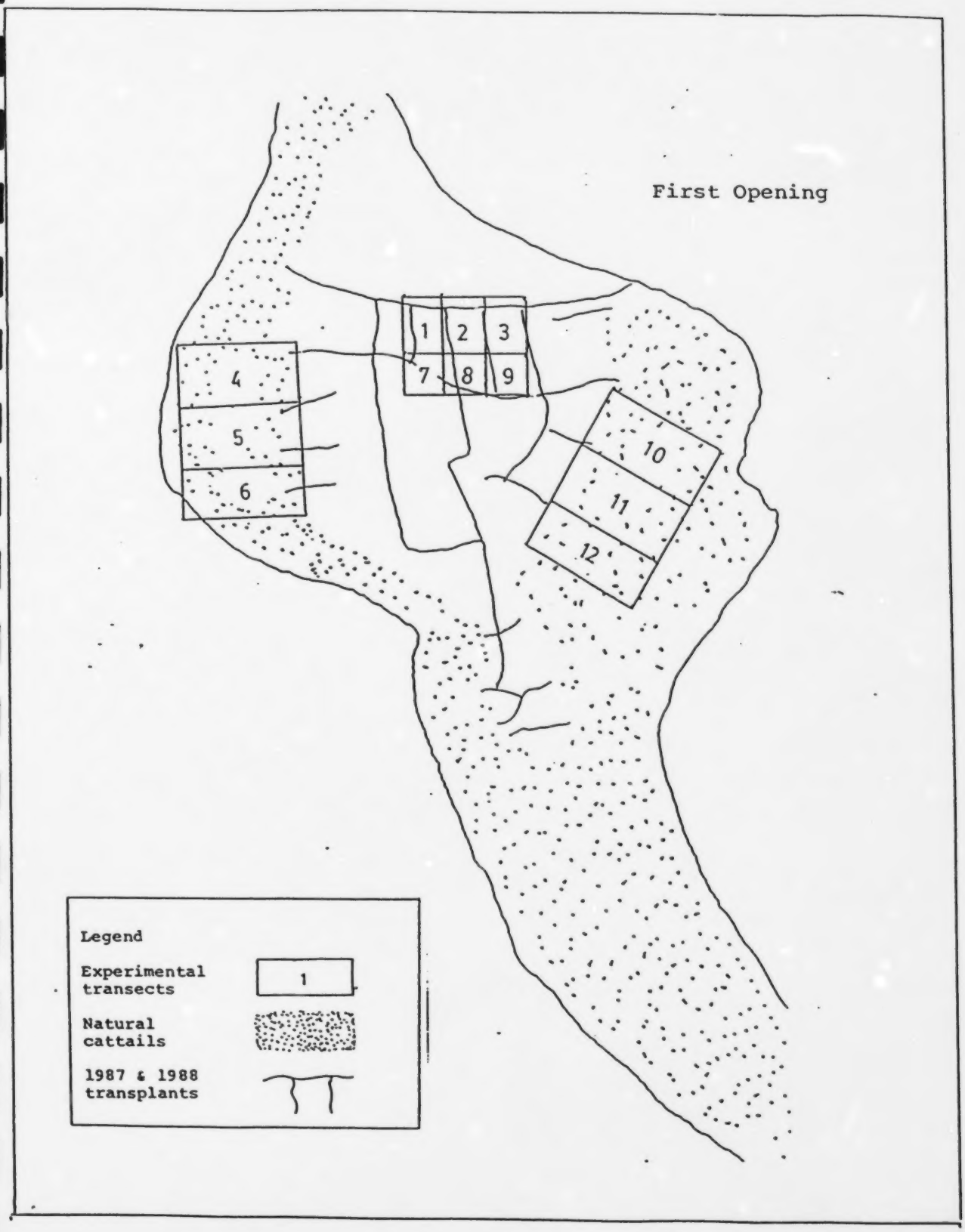
To determine the different effects of both fertilizers, two different concentrations were used. 20:1 and 10:1 concentrations were applied to select areas of cattail stands.

In total, three different areas were chosen for the application of Foliar fertilizer. Two of these areas were natural cattail stands while the third was a transplanted section. One of the natural areas received a 10:1 concentration of both the 14-4-6 and 4-18-6 fertilizers in two separate transects. A third transect in each area was used as a control and was untreated (See Map 2, Tables 1,2).

The transplanted area contained six transects, four of which contained the two different fertilizers (14-4-6, 4-18-6) at both concentrations (10:1, 20:1). The remaining two transects were designated as controls and remained untreated.

Each transect in the natural cattail stand was approximately 75 square metres and received 0.2 L/m^2 of the appropriate fertilizer in dilutions of both 20:1 and 10:1, while each transect in the transplanted cattail plot was approximately 20 square metres and received 0.25 L/m^2. The fertilizer was applied using a backpack fire pump.

To determine the effects of the applied fertilizer, samples of cattail leaves were taken from each transect within an area after a two to three week waiting period. The leaves were examined for any detrimental effects such as burning.



Map 2: Experimental Transect Locations (First opening).

Table 1: Fertilizer Application: Leaf Observations (Natural Cattails)

Application N-P-K	Transect Conc.	Test Site #	Sample #	% Chemical Coverage	Tip Burn (mm)	% Burn on Whole Leaf	Colour of Burn	Burn Size (mm)	Burn\Chemical Coverage (%)
14-4-6	20:1	10	1	25	22	0.001	Br-Bl	0.5 - 1.0	0.004
			2	10	45	0.001	Br	1.0 - 4.0	0.01
			3	2	23	0.0001	Br	0.2	0.005
			4	30	18	0.01	Dbr	0.5 - 1.0	0.033
			5	2	20	0.001	Dbr	0.5	0.05
			6	80	38	0.01	Dbr	0.1 - 1.0	0.0125
			7	10	22	0.01	Dbr	0.1 - 3.0	0.1
14-4-6	10:1	4	1	10	34	5	Dbr	0.1 - 10.0	50
			2	80	55	1	Dbr	0.1 - 10.0	1.25
			3	20	48	2	Dbr-Lbr	0.01 - 3.0	10
			4	5	40	2	Dbr-Lbr	0.1 - 8.0	40
4-18-6	10:1	6	1	20	50	20	Y LBr	0.1 - 5.0	100
			2	10	40	10	YW	0.1 - 3.0	100
			3	1	22	0.5	YW	0.01 - 5.0	50
			4	2	18	1	YW	0.1 - 2.0	50
4-18-6	20:1	12	1	0.1	18	0.0001	YW	0.01 - 0.1	0.1
			2	1	20	0.001	YW	0.01 - 0.1	0.1
			3	0.1	25	0.01	YW	0.01 - 2.0	10
			4	0.1	15	0.01	YW	0.01 - 1.0	10
			5	1	13	2	YW	0.01 - 10.0	200
Control		11	1	0	12	< 0.001	Br @ tip	0.1	-
			2	0	15	< 0.001	Br @ tip	0.1	-
			3	0	15	< 0.001	Br @ tip	0.1	-
			4	0	18	< 0.001	Br @ tip	0.1	-
			5	0	20	< 0.001	Br @ tip	0.1	-

Legend:

Br.- brown
Bl.- black
DBr.- dark brown
LBr.- light brown
BrW.- brownish white
Y.- yellow
YW.- yellowish white

* See Map 2 for Test Site locations.

Table 2: Fertilizer Application: Leaf Observations (Natural (Transplanted Cattails)

Application N-P-K	Transect Conc.	Test Site #	Sample #	% Chemical Coverage	Tip Burn (mm)	% Burn on Whole Leaf	Colour of Burn	Burn Size (mm)	Burn\Chemical Coverage (%)
4-18-6	10:1	3	1	5	18	5	BrW	0.1 - 5.0	100
			2	5	25	10	BrW	0.1 - 5.0	200
			3	0.1	40	0.1	BrW	0.1 - 2.0	100
			4	1	10	1	BrW	0.1 - 1.0	100
			5	1	20	1	BrW	0.1 - 3.0	100
4-18-6	20:1	9	1	10	80	15	Y	spread	150
			2	2	60	5	BrW	0.1 - 1.0	250
			3	2	85	5	BrW	0.1 - 5.0	250
			4	5	20	5	BrW	0.1 - 6.0	100
			5	10	40	30	BrW	0.1 - 10.0	300
14-4-6	10:1	1	1	40	1	0.5	DBr	1.0 - 5.0	1.25
			2	50	50	5	DBr	0.1 - 3.0	10
			3	35	80	1	DBr	0.1 - 3.0	2.86
			4	55	10	0.5	DBr	0.1 - 5.0	0.91
14-4-6	20:1	7	1	70	5	0.1	DBr	0.1 - 1.0	0.143
			2	50	10	0.1	DBr	0.1 - 1.0	0.2
			3	35	5	0.5	DBr	0.1 - 0.2	1.43
			4	55	2	1	DBr	0.1 - 1.0	1.82
Control		2	1	0	17	0	-	-	-
			2	0	18	0	-	-	-
			3	0	10	0	-	-	-
			4	0	20	0	-	-	-
			5	0	20	0	-	-	-

Legend:

Br.- brown
Bl.- black
DBr.- dark brown
LBr.- light brown
BrW.- brownish white
Y.- yellow
YW.- yellowish white

* See Map 2 for Test Site locations.

4.0 Results

In both the natural and transplanted cattail stands, the 4-18-6 fertilizer displayed a much greater degree of burning than the 14-4-6 fertilizer in both the the 10:1 and 20:1 dilutions. The difference in area burned was especially noticable for the 20:1 dilution, where area of leaves burned for 14-4-6 was negligible compared to the 4-18-6 treatment (refer to figure 2,3, plates 1-10).

In the natural cattail stands, the 10:1 dilution showed a higher degree of burning than the 20:1 for both the 14-4-6 and 4-18-6 fertilizers (refer to figure 2). However, in the transplanted cattail stands the 20:1 dilution of 4-18-6 showed a considerably greater extent of burning than the 10:1 dilution (refer to figure 3).

Average percent burn for the transplanted cattails exceeded chemical coverage for both 10:1 and 20:1 dilutions of the 4-18-6 fertilizer (refer to table 2).

Cattails sprayed with 14-4-6 N-P-K foliar fertilizer displayed a burn of a dark colouration, while those sprayed with 4-18-6 showed burns of a lighter colour (refer to tables 1,2).

In most cases, the 14-4-6 fertilizer showed greater tip burn than the 4-18-6 , except for the 20:1 dilution in the transplant cattail stand where the 4-18-6 fertilizer had an average tip burn 10 times greater than the 14-4-6. All controls also showed a significant amount of tip burn (refer to figures 4,5, tables 1,2).

FIGURE 2: PERCENT OF CHEMICAL COVERAGE SHOWING BURN
NATURAL CATTAIL STAND

FROM 14-4-6 AND 4-18-6 NPK FERTILIZER (10:1 AND 20:1 CONCENTRATIONS)

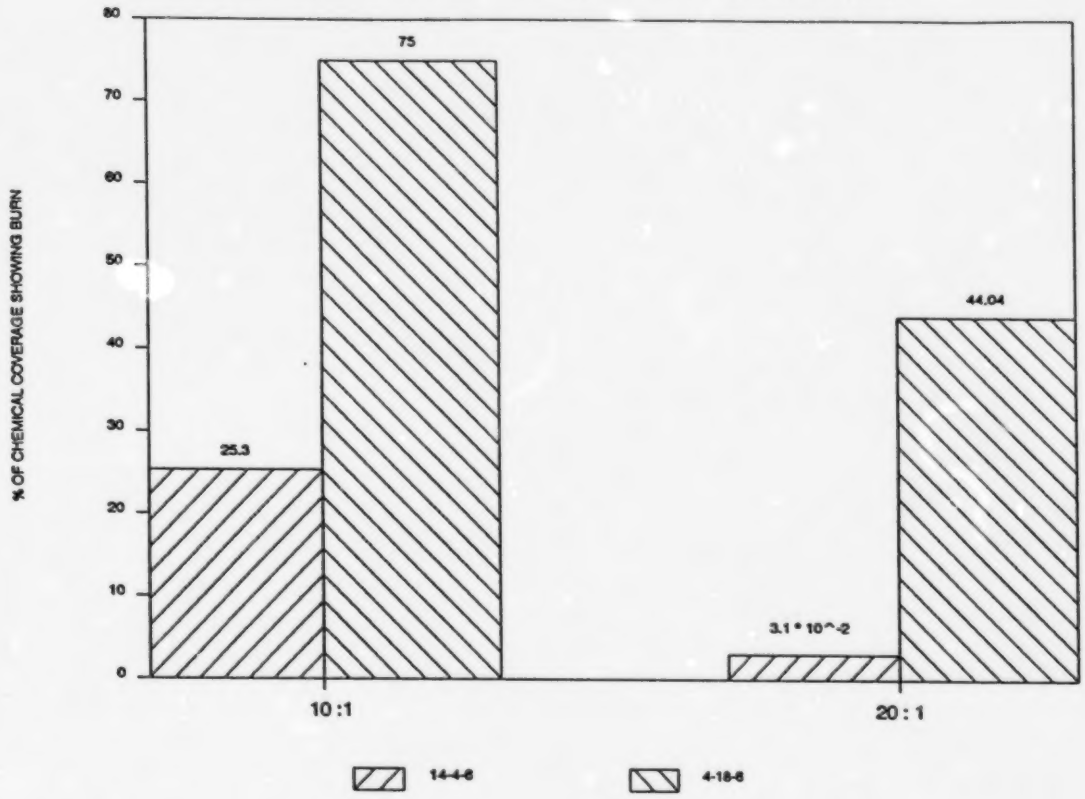


FIGURE 3: PERCENT OF CHEMICAL COVERAGE SHOWING BURN
TRANSPLANT CATTAIL STAND
FROM 14-4-6 AND 4-18-6 NPK FERTILIZER (10:1 AND 20:1 CONCENTRATIONS)

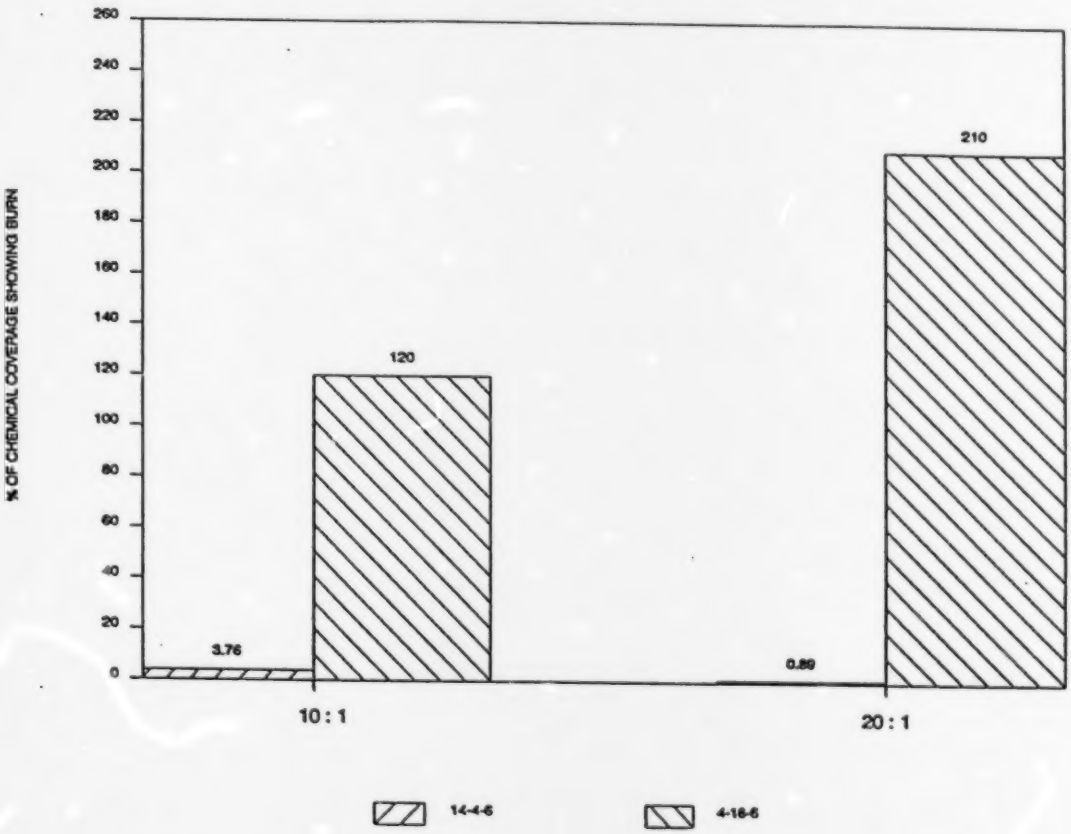


FIGURE 4: AVERAGE TIP BURN IN NATURAL CATTAIL STAND

14-4-6 AND 4-18-6 FERTILIZERS (10:1 AND 20:1 CONCENTRATIONS)

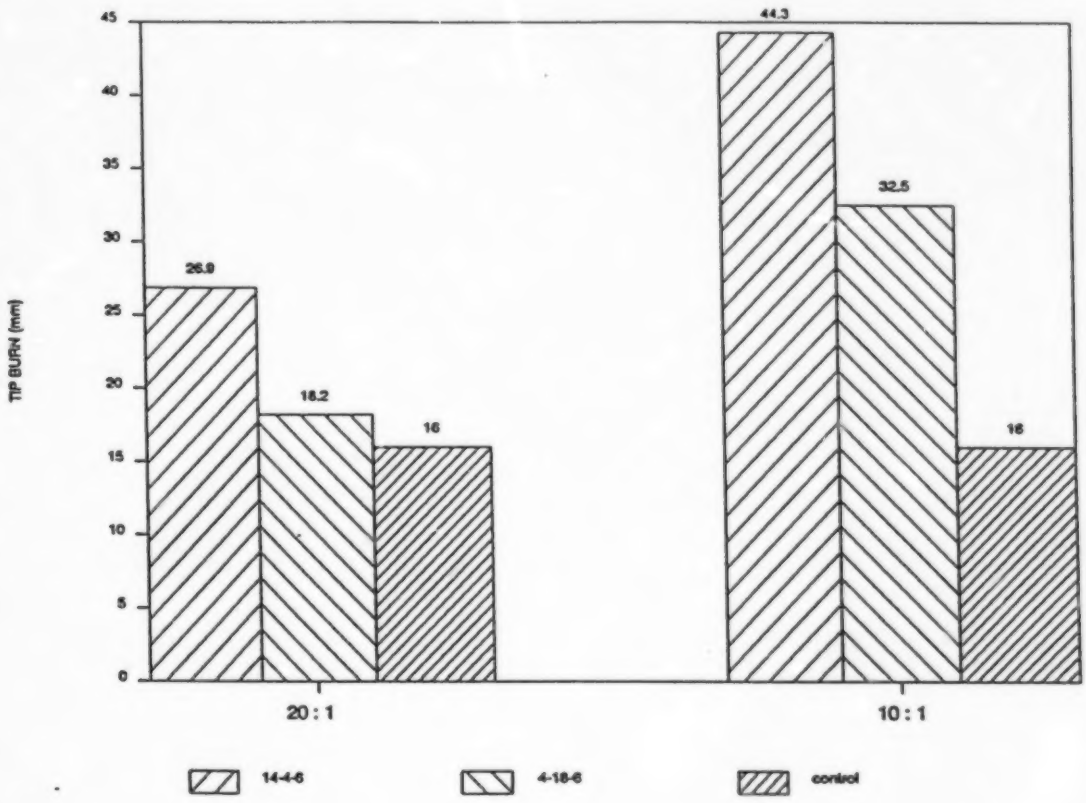
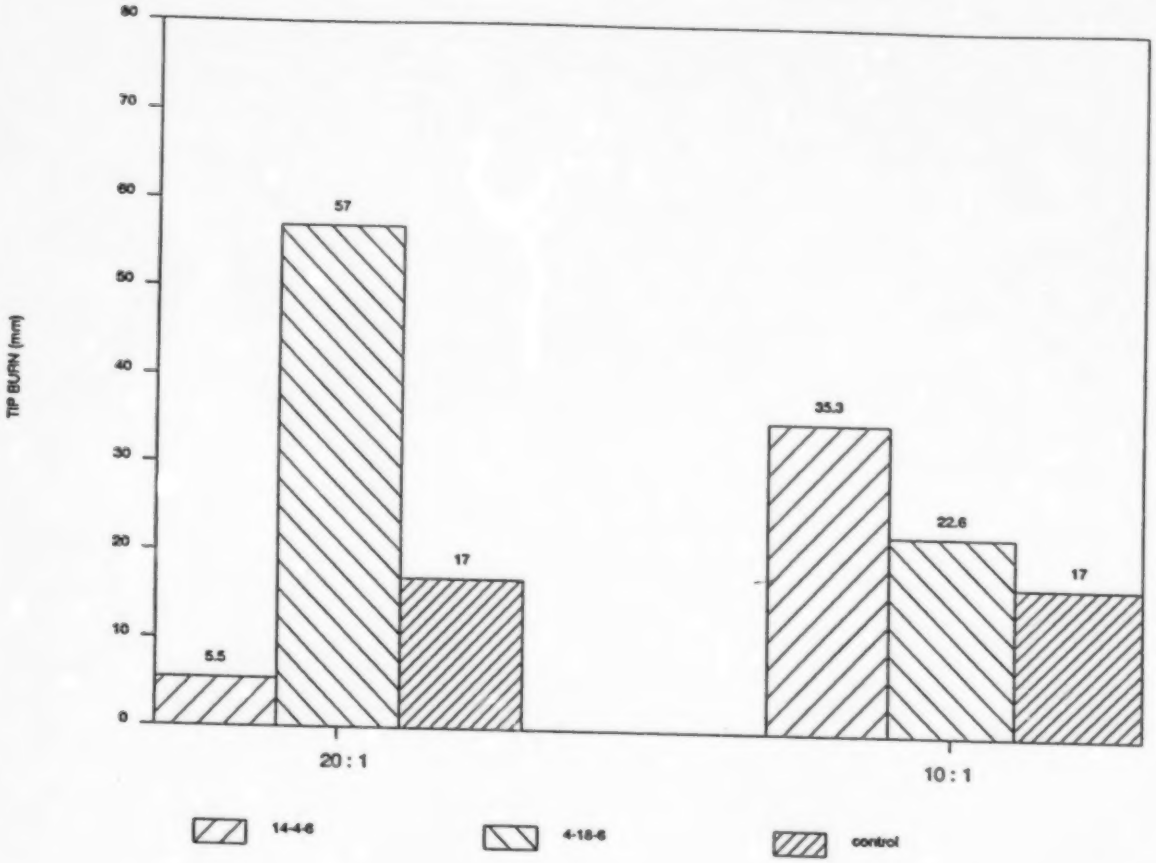


FIGURE 5: TIP BURN TRANSPLANT CATTAIL STAND

14-4-6 AND 4-18-6 FERTILIZERS (10:1 AND 20:1 CONCENTRATIONS)



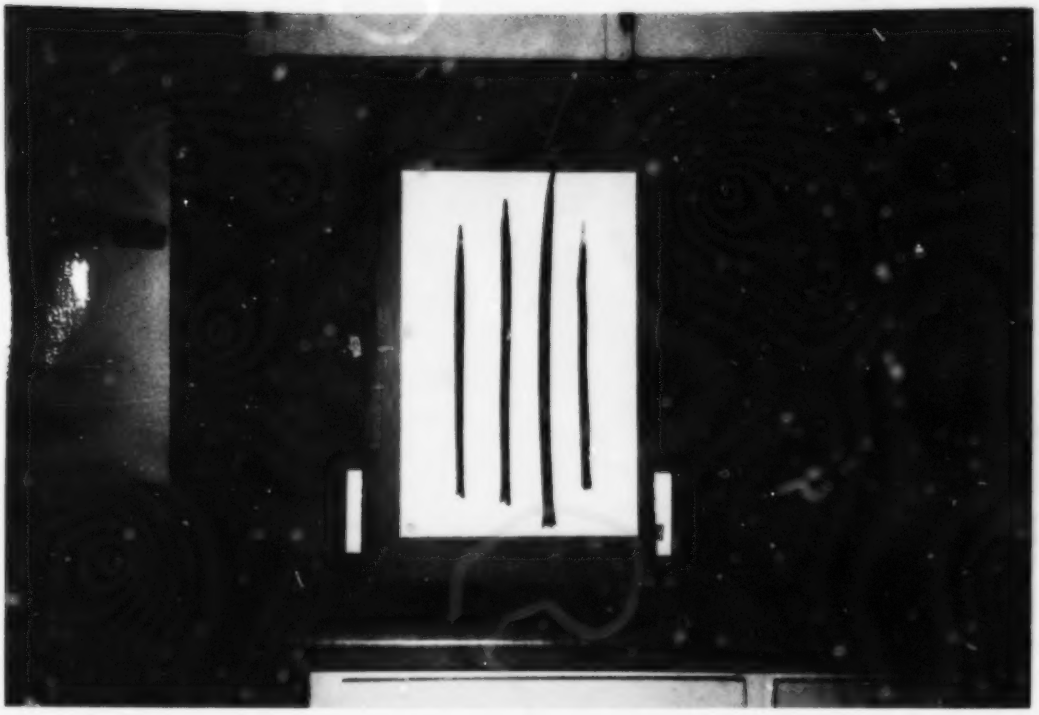


Plate 1: Transplant Cattail Stand (4-18-6, 10:1)

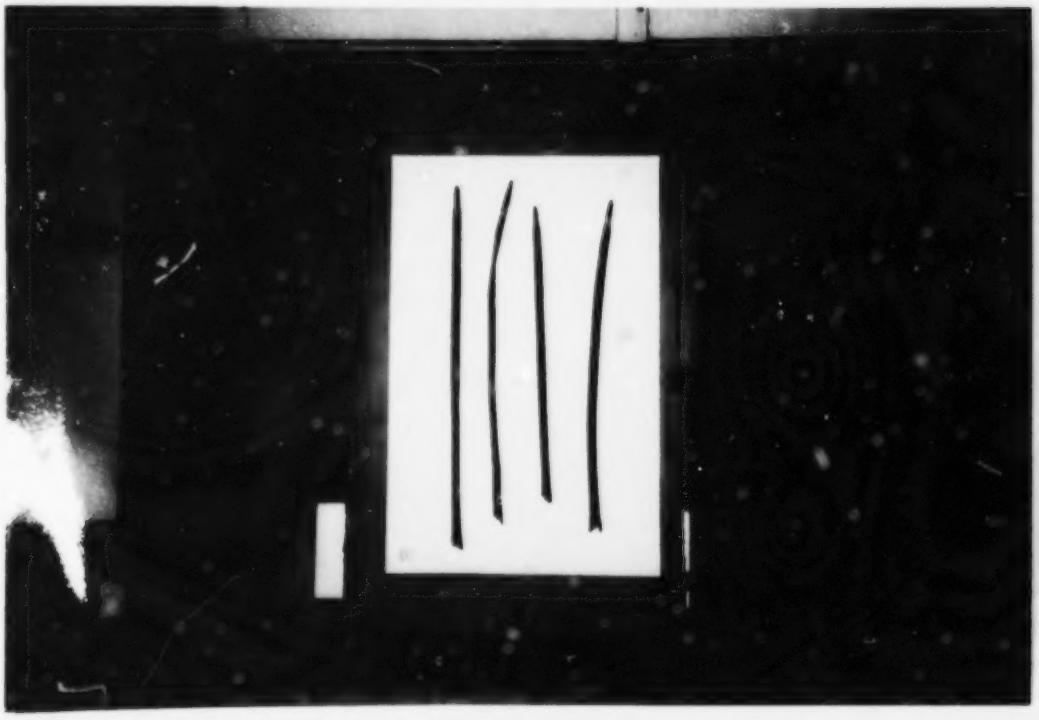


Plate 2: Transplant Cattail Stand (14-4-6, 10:1)

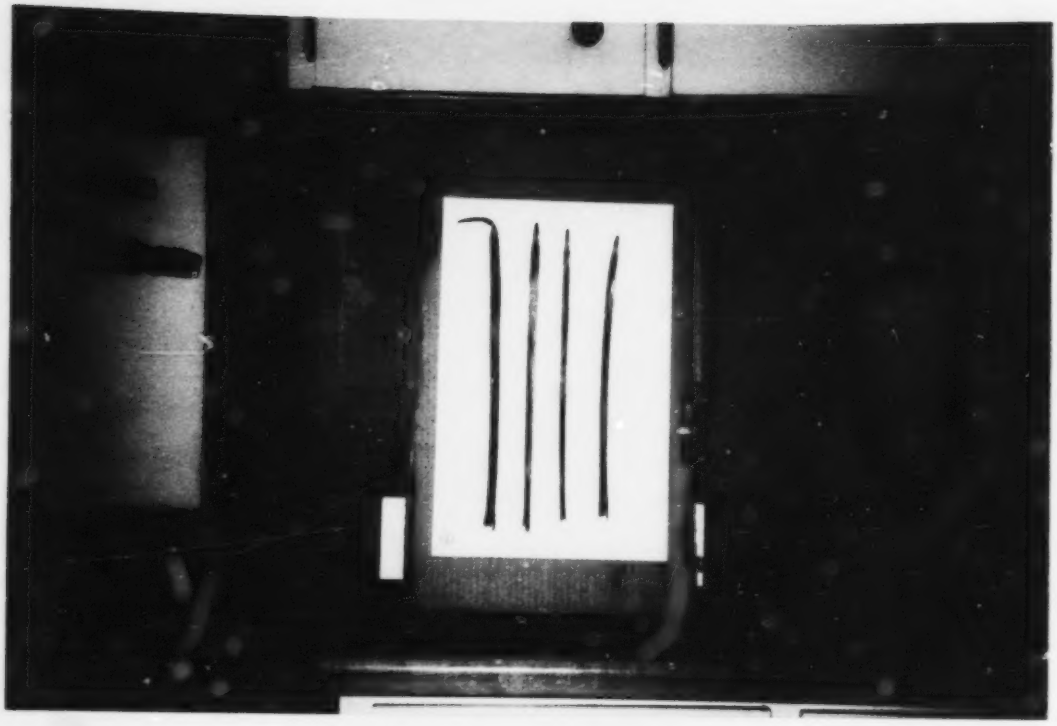


Plate 3: Transplant Cattail Stand (4-18-6, 20:1)

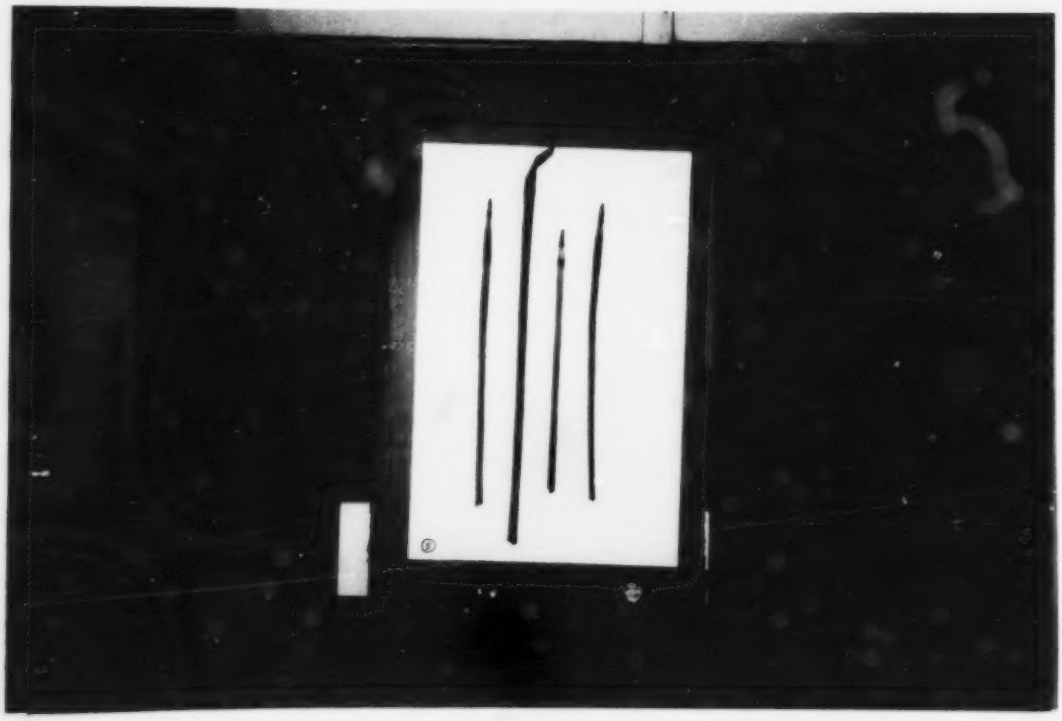


Plate 4: Transplant Cattail Stand (14-4-6, 20:1)

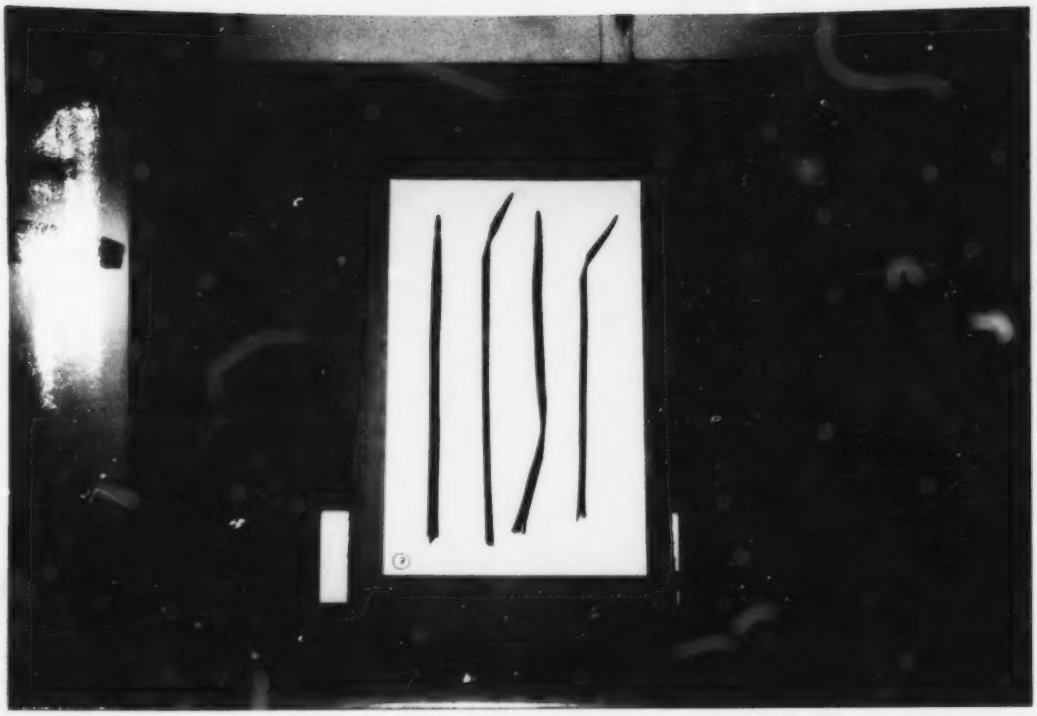


Plate 5: Transplant Cattail Stand (control)

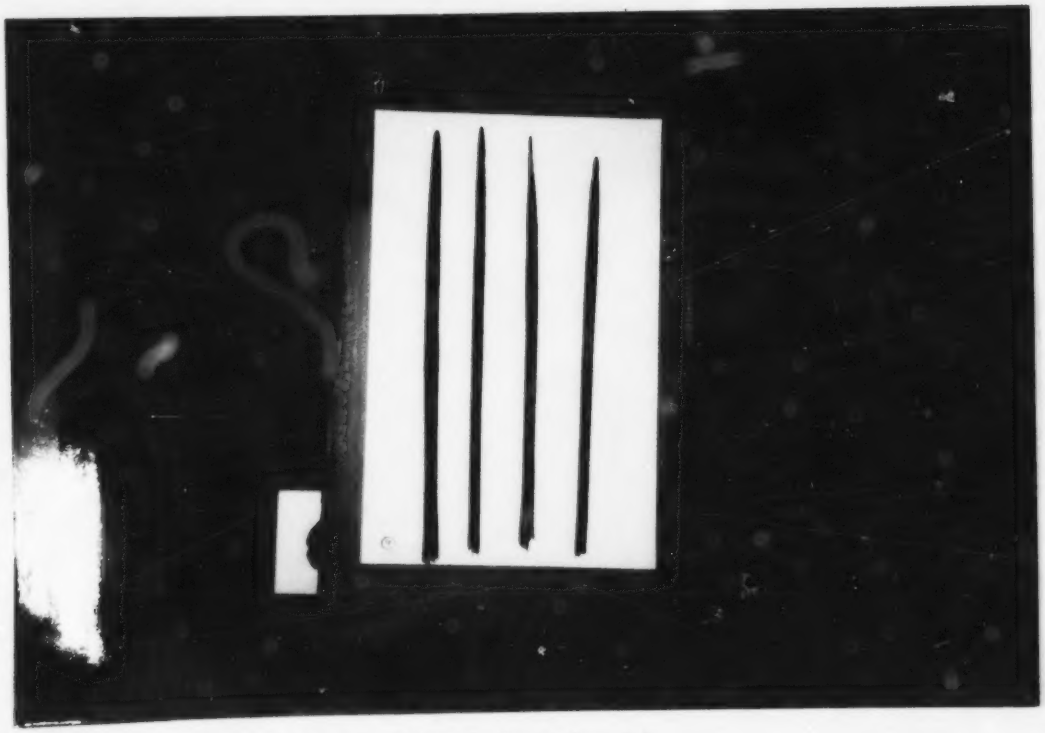


Plate 6: Natural Cattail Stand (control)

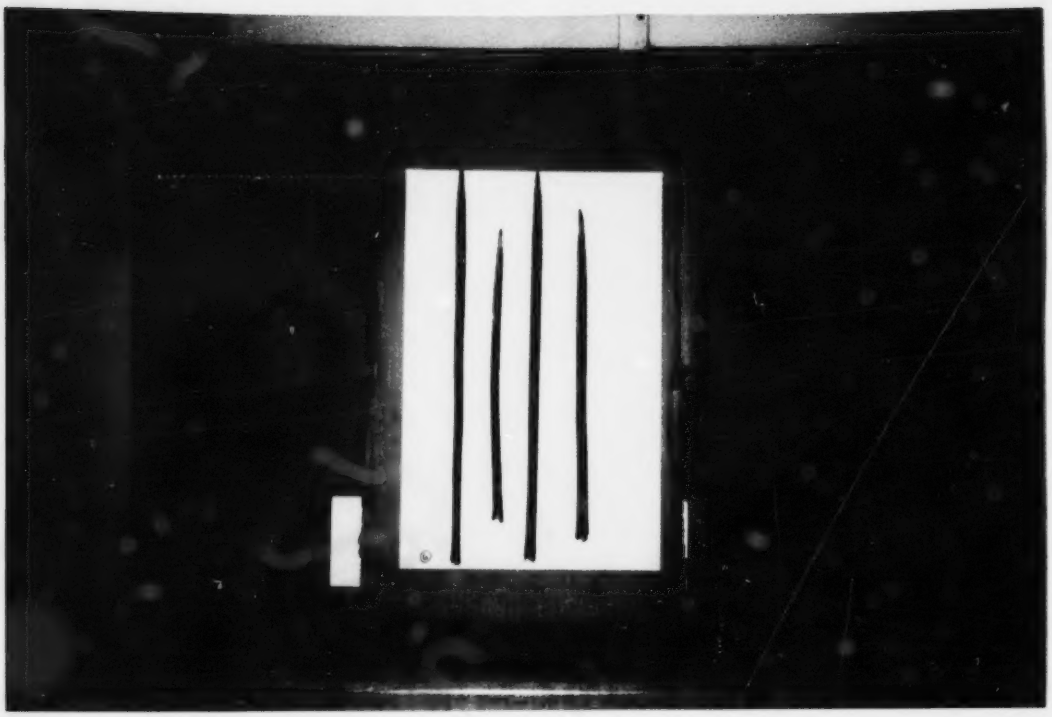


Plate 7: Natural Cattail Stand (4-18-6, 10:1)

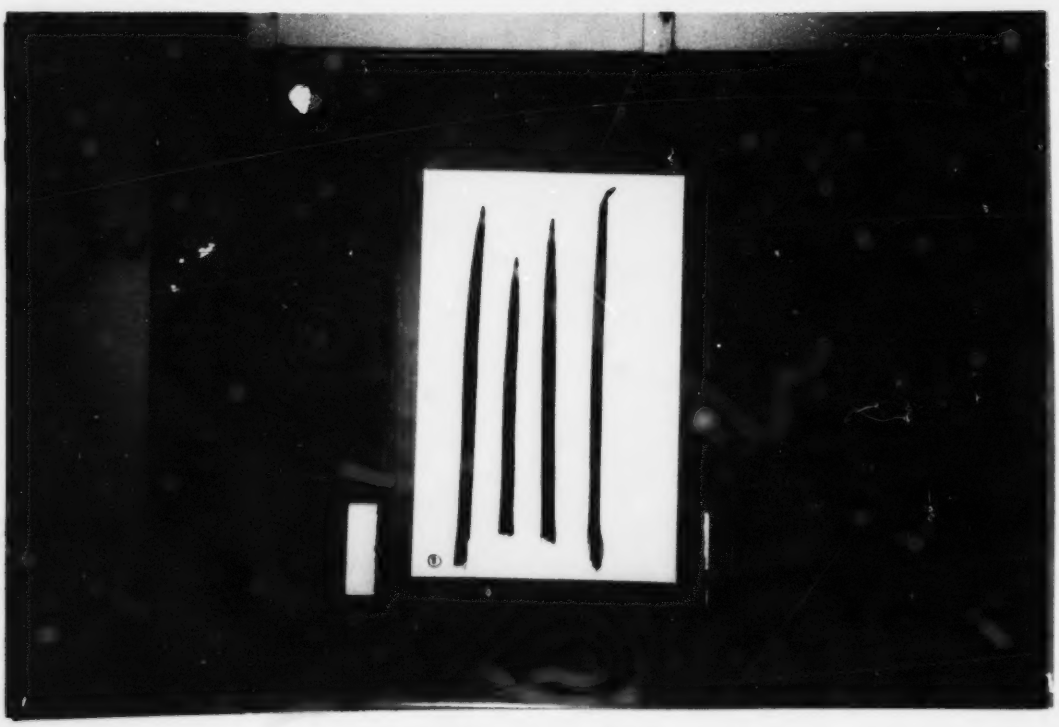


Plate 8: Natural Cattail Stand (14-4-6, 10:1)

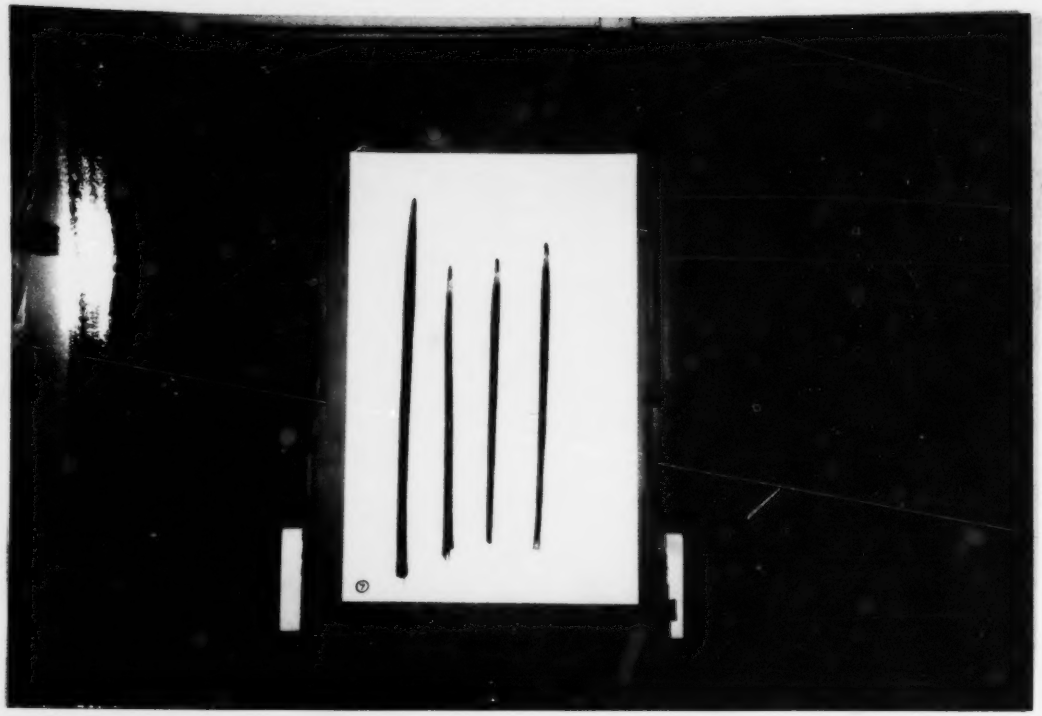


Plate 9: Natural Cattail Stand (4-18-6, 20:1)

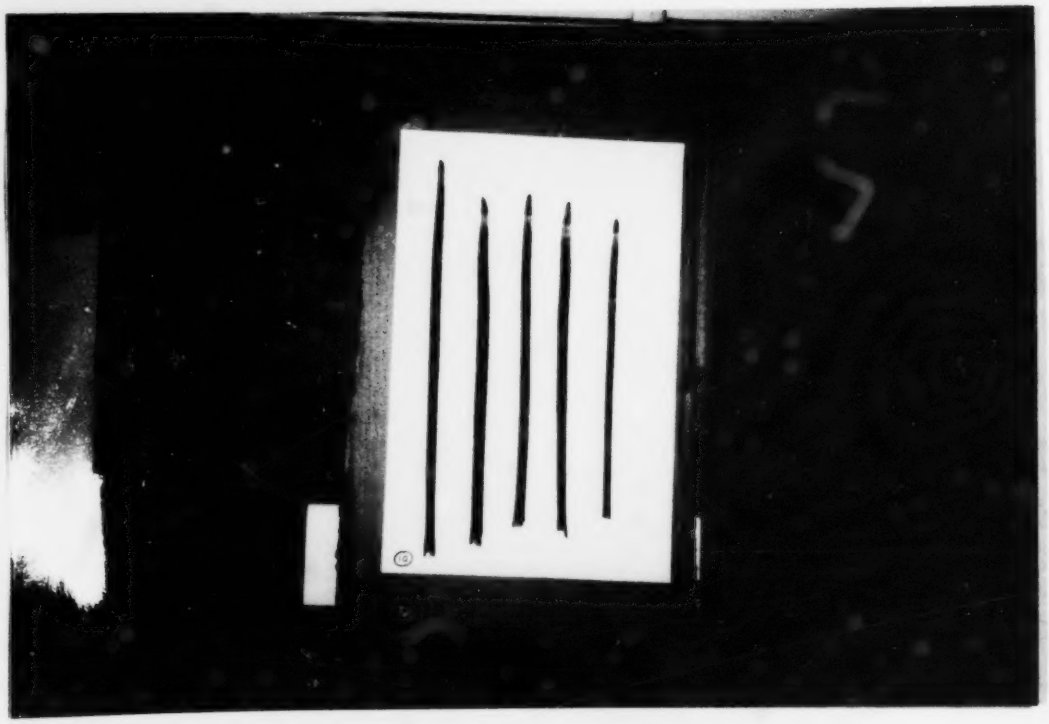


Plate 10: Natural Cattail Stand (14-4-6, 20:1)

5.0 Discussion and Conclusions

From the results of this study it can be concluded that the 4-18-6 N-P-K fertilizer burned the cattail leaves to a greater extent than the 14-4-6. However, these burns were of a lighter colour than those that appeared on the leaves of the 14-4-6.

In the case of the natural cattails the 10:1 dilutions displayed a greater amount of burning. The 20:1 dilution, on the other hand, displayed more burning in the transplanted cattail stands than did the 10:1. However, it can not be concluded that these burns are due directly to the application of the fertilizer. Other variables such as plant disease, burning due to the tailings and burning caused by the dilution water used (pH 3.0) could have possibly been a contributing factor. This is further illustrated when examining the results from the transplanted cattails. The average percent burn exceeded the average chemical coverage. This would indicate outside interference from another source and could possibly explain why the 20:1 dilution burned more than the 10:1.

With respect to tip burn, it was decided that no direct correlation existed between fertilizer application and the tip burn. In all other cases the burn occurred within a certain chemical coverage. Since no chemical coverage existed on the tip, the burns are probably caused by another factor. The fact that in one case the control cattails displayed a higher degree of tip burn strengthens this conclusion.

6.0 Recommendations

From results obtained, it was determined that the application of Foliar fertilizer should continue, but before any solid conclusions can be drawn as to the effects of the fertilizer, a thorough study must be conducted. The following are a number of recommendations to be used for future experiments. These recommendations should increase the odds of achieving the desired end result, namely a larger survival rate of cattails with an increased biomass.

1. Foliar fertilizer application rate is currently 0.2 - 0.25 L/m^2 with dilutions of 10:1 - 20:1. *Stoller Chemicals* recommend an application rate of 0.02 L/m^2 and a dilution of 80:1.

2. 14-4-6 and 4-18-6 N-P-K fertilizers should be tested together as well as separately to see if they complement one another. *Stoller Chemicals* recommends the use of 14-4-6 *Harvest Plus* foliar fertilizer early in the spring to provide necessary early plant vigour, followed by 4-18-6 after fruiting to provide improved root development, size, uniformity and maturity.

3. Calcium should be applied along with the foliar fertilizer. *Harvest Plus* can accelerate the rate of leaf growth to a point

beyond the root's ability to supply calcium from the soil. Since calcium is immobile in the plant, and because it is directly related to cell division, cell expansion, and cell to cell adhesion, calcium must be made available as rapid tissue development occurs. 1 litre of calcium should be applied for every 2 litres of foliar fertilizer.

4. Fertilizer application should begin 4-8 weeks into growing season and proceed on a weekly basis for about 8 weeks. Biomass and nutrient accumulation are in lag phase during the first 4-8 weeks of spring. However, the plant then enters a rapid growth phase in which 47-80% of the total seasonal biomass production and nutrient uptake occurs in a 4-8 week period. During this time, Typha leaves account for 60-70% of the biomass and represent the major nutrient sink (Garver, 1988).

5. Control quadrats should be sprayed with dilution water in the future to ensure that burning is in fact caused solely by the foliar fertilizer, and not the water. *Stoller* claims that high salt content, not acid causes leaf burning, and therefore, their fertilizer (which is low in salt) will not cause foliage burn. If foliage burn is extensive enough, photosynthetic activity may be affected.

6. In the future experimental quadrats should be positioned

further apart from other quadrats to ensure no cross contamination occurs.

7.0 References

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SECTION 2: Summary paper of our understanding of the microbial alkalinity generation to date

TUESDAY AUGUST 9, 1989 (CONTINUED)

BIOREACTOR TECHNOLOGY

3:00 P.M.	3:30 P.M.	M. Kalin (Canada)
3:30 P.M.	4:00 P.M.	G. Andrews, INEL
4:00 P.M.	4:30 P.M.	J. Gaddy, University of Arkansas
4:30 P.M.	5:00 P.M.	Open Discussion

WEDNESDAY, AUGUST 9, 1989

MOLECULAR BIOLOGY AND ENZYMOLOGY

8:00 A.M.	8:30 A.M.	Continental Breakfast
8:30 A.M.	9:00 A.M.	F. Roberto, INEL
9:00 A.M.	9:30 A.M.	N. C. Mishra, University of South Carolina
9:30 A.M.	10:00 A.M.	J. Kitchell, Holometix, Inc.
10:00 A.M.	10:15 A.M.	BREAK
10:15 A.M.	10:45 A.M.	J. Dordick, University of Iowa
10:45 A.M.	11:15 A.M.	W. Wood, Salk Institute

MICROBIAL SOLUBILIZATION OF COAL

11:15 A.M.	11:45 A.M.	R. M. Fakoussa (West Germany)
11:45 A.M.	12:15 A.M.	D. Crawford, University of Idaho
12:15 P.M.	2:00 P.M.	LUNCH D. Beecy, US DOE

PANEL MEETINGS

2:00 P.M.	4:30 P.M.	Meetings
7:00 P.M.	9:30 P.M.	DINNER

ECOLOGICAL ENGINEERING METHODS FOR ACID MINE DRAINAGE TREATMENT OF COAL WASTES

M. Kalin, Boojum Research Limited,
Toronto, Ontario, Canada

J. Cairns, Dearborn Environmental Consulting Services,
Mississauga, Ontario, Canada

R. McCready, Energy Mines and Resources, CANMET,
Ottawa, Ontario, Canada

ABSTRACT

The treatment of acid mine drainage through the utilization of alkali generating microbes has potential as an alternate approach to conventional lime treatment. Organic matter, a source of fixed carbon for the alkali generating microbial ecosystem, has been tested in 6 different types of AMD. The AMD characteristics range in acidities from 2 mg/l to 900 mg/l ($CaCO_3$ equivalent), while sulphate concentrations range from 75 to 7300 mg/l. Alkali generating populations identified include iron reducers, sulphate reducers and ammonifiers. In coal AMD amended with organic matter, the microbial alkali generation is dominated by ammonifiers. Concentrations of Al, Fe and Zn in the AMD water decreased concurrent to increases in pH (3.2 to 6.5) in localized areas in the test cells.

INTRODUCTION

Acid mine drainage (AMD) leaving coal and pyritic base metal operations presents a major environmental problem. Treatment methods generally require the addition of lime to increase the pH, decrease the acidity and thereby precipitate heavy metals. Construction of wetlands in AMD drainage or seepage areas from pyritic waste sites, an alternate approach to the lime treatment, is currently receiving attention (Hedin et al., 1988; Skousen and

Sencindiver, 1988; Dollhopf et al., 1988; Mitsch and Jorgensen, 1989).

Wetlands are complex ecosystems which include a diversity of microbial activities which can generate a natural source of alkali. The alkali is the product of several processes, microbial reducti of sulphate and iron, denitrification and methanogenesis (Herlihy and Mills, 1985;Herlihy et al., 1987; Williams and Crawford, 1983; Gould and McCready, 1982). The effectiveness of these processes as an AMD treatment system will be dependent on the rate at which they generate alkali. For example, sawdust has been reported to increase sulphate reduction rates (King et al., 1974; Jongejan, 1986; Tuttle et al., 1969a,b). Determination of the rates of these processes are typically examined upon isolation of specific microbial populations. Therefore, these rates are only partially indicative of those occurring in the natural environment. Moreover, in nature, their interaction may be synergistic, antagonistic, or syntropic.

As, at present there is limited knowledge of these interactions in natural environments, the effective utilization of wetlands for the treatment of AMD is extremely difficult (Baas-Becking, 1925, Trudinger et al., 1979). Furthermore, little is known of the effect of elevated sulphate, iron, and heavy metal concentrations, all present in AMD at levels exceeding those found in naturally acidic wetlands by several orders of magnitude. The source(s) of organic carbon is of primary importance to microbially generated alkali in ecosystems receiving acid mine drainage.

Microbial populations found in association with straw placed in different AMD streams are described and alkali generating bacteria groups are identified.

METHODS AND MATERIALS

Description of experimental test areas

All tests were carried out at mine sites located in the provinces of Ontario and Nova Scotia, Canada. Each test area differs by conditions under which the AMD is generated in association with pyritic wastes.

Pyrrhotite tailings AMD: At a site in Ontario, acidic seepage was pooled behind three small dams constructed on a pyrrhotite tailings area generated by a nickel mining operation. Each pond was separated into three zones, to which peat and hay, straw and sawdust, and weathered overburden, respectively, were added. Placement of the organic amendments was carried out in June of 1986; in the summer of 1987, the hay and straw was repeated. The seepage pools' turnover times, driven by run-off, are very long.

Uranium tailings AMD: AMD seepage collects in a small pond (0.15 ha) at the foot of an abandoned uranium tailings dam located in Ontario. Water levels reach as high as 1 m in the spring, but severely decline during summer months, leaving a moist area with

little water coverage. Straw was placed in this pool in the spring of 1987 and 1988.

Copper-zinc waste rock AMD: A pond of about 0.5 ha receives AMD from a waste rock pile and run-off from zinc and copper concentrate storage areas. Standing water is present throughout the year, while pond overflow occurs in spring and fall. In 1987, sawdust was placed in the area adjacent to the overflow weir to a thickness of 1 m. In 1988, sawdust was thinly spread over the entire pond, while straw was placed adjacent to the overflow weir.

Copper-zinc tailings AMD: Acidic seepage drains from the beach of a decant pond located on a 40 ha tailings area covered with revegetated overburden layer. Straw was placed in the summer of 1988 in the seepage along the tailings beach.

Coal AMD: Regions of a sphagnum/leatherleaf bog have withstood acid mine drainage from a coal washing plant and from a Lifting and Banking Centre (LBC) in Cape Breton, Nova Scotia for about 5 years. Straw was implanted to the depth of the root zone of the heavily stressed vegetation cover in August 1988. A portion of the AMD flow was diverted into a second, previously unstressed bog within the same drainage basin in order to study the immediate effects of AMD damage. Organic material, collected from the second bog for the microbial investigation, had accumulated in an area which had experienced AMD stress for 4 months.

Organic matter placement and collection

Sawdust was placed by back-hoe or dumped from a truck into the AMD test areas. All straw/hay and peat amendments were distributed as evenly as possible by hand.

Amendment samples were collected by submerging a 2 litre wide-mouth plastic container into the amendment layer and filling the container with amendment and water. The containers were immediately sealed, and transport to the laboratory where they were refrigerated until the microbiological sub-samples were withdrawn for tests.

Water collection and analysis

Water samples collected from the amended locations were filtered (0.45 um pore size) and acidified with nitric acid within 24 h. Samples were submitted to a certified lab for determination of the concentrations of major elements using Inductively Coupled Plasma Spectrophotometry. Following gentle aeration with CO_2 to remove hydrogen sulphide, sulphate concentrations were determined by Ion Chromatography (Dionex). The sample was filtered through a 0.45 micron filter prior to analysis. Acidity was determined using the ASTM method D 1067-88-B.

Microbiological methods

All microbiological analyses were initiated within two days of sample arrival at the laboratory. Analyses were conducted using

liquid portion of the sample, following shaking of the whole sample.

ATP (Adenosine Triphosphate) Assays: ATP was measured by the Firefly Luciferase method using a Turner Designs Model 20e design photometer. The technique used to extract ATP from the microorganisms proved to be successful for the AMD samples. After lysing the cell walls with ethanol, the ethanol was removed by filtration through a glass fibre filter. The cells were further lysed by passing acetone through the filter. Finally, ATP from the organisms in the filter was leached with trisodium phosphate buffer. The extracts were further diluted in trisodium phosphate buffer containing Mg-EDTA in order to prevent inhibition of firefly luciferase due to metal ions.

Estimation for Sulphate Reducing Bacteria (SRB): Five different modifications of SRB media were used. Firstly, Postgate B with titanium as a reducing agent was used. The same medium, but adjusted to pH 6.0, constituted the second medium. Third, Postgate B with acetate substituted for lactic acid as the carbon source; fourth, Postgate E (similar to Postgate B, but contains agar), and fifth, Postgate F (adjusted to pH 6). Levels of sulphate reducing bacteria, iron reducing bacteria and ammonifiers were estimated by end-point dilution in selected media, prepared in a decimal series.

Estimation of ammonifying and iron reducing bacteria: Postgate E and F media were used to enumerate sulphate reducers, while casein medium (Microbiological Analysis of Waters, Wastewaters, and Sediments, Canada Centre for Inland Waters, 1978) was used for ammonifiers. Iron reducing bacteria were enumerated in a minimal salt medium containing Fe III and a mixture of peptone, yeast extract and lactate as a carbon source. The yeast extract also served as a supply growth factor. All cultures were incubated for three weeks at 28° C.

RESULTS

Field results

Sampling of amendments for the determination of the microbial ecology was preceded by field observations and measurements of pH in the amendment layers. These results are reported in detail for the pyrrhotite and coal AMD.

Initial tests using organic matter amendments were performed in pyrrhotite AMD in 1986. Within 2 weeks of amendment addition, pond water, initially stained red with ferric ions, became colourless due to reduction of iron to the ferrous state. This indicated that the organic amendment stimulated microbial iron reduction. After about 1 month after organic matter addition, local pH increases from 3.0 to 4.4 were recorded within dense pockets at the base of the amendment layer. Examination of samples taken within these pockets for a preliminary microbial

investigation confirmed the presence of sulphate reducers. During sampling in January, 1988, upon penetration of the overlying ice layer, a strong smell of hydrogen sulphide was detected. Cairns et al. (1988) identified in these samples the presence of those microbial groups in the organic amendment which can explain the alkali generation. Given that AMD characteristics will play an important role in the microbial ecology, organic matter was placed in different types of AMD. In Table 1, the characteristics of AMD solutions collected with the organic matter is presented.

The concentrations of free mineral acidity in the water of the test areas ranged between 2 mg/l in the Cu-Zn tailings AMD, to 900 mg/l in those samples from the uranium tailings AMD. The pH ranged between a value of 6.0 in the bog which received coal AMD for 5 years and a pH value of 2.6 in the uranium AMD. As expected, the Cu and Zn concentrations from the Cu-Zn waste-rock AMD are high, with 226 and 36 mg/l, respectively. In the coal AMD, both metals are low with <0.01 mg/l. The iron concentrations range between 1 mg/l in the Cu/Zn tailings to 1900 mg/l on the pyrrhotite AMD. The sulphate concentrations are highest in the pyrrhotite AMD, with concentrations of 7300 mg/l, while concentrations as low as 300 mg/l determined in samples taken from the bog receiving coal acid mine drainage. Concentrations of sulphate in freshwater range from 2 to 150 mg/l (Allen et al., 1974). The concentration of sulphate in the bog which has received acid mine drainage for 4 months (75 mg/l) is in the range of fresh water. All experimental test areas exhibit pH pockets as described for the pyrrhotite AMD. The AMD

characteristics described in Table 1 would suggest that the most favourable conditions for microbial alkali generation are present in the coal AMD.

A relationship between the increased pH and water quality changes was only apparent in coal AMD to date. The minimum and maximum pH values and concentrations of Al, Fe and Zn are presented in Table 2 for water collected in May and June, 1988. After placement of straw in August, 1988, the test cells with amendment showed a surface film on the water by May, 1989. Water underneath the film had consistently higher pH value of than water without film (Table 2). The concentrations of Ca, K, Mg, Mn, Na, Ni, P, S and Si did not differ in water with or without film or amendment. However, concentrations of Al, Fe and Zn in the water covered by film were considerably lower than those in waters without a film or amendment.

Microbial Ecology

The AMD characteristics (Table 1) are significantly different between the test sites. Therefore, it can be expected that the microbial ecology will be affected. Notable differences between the samples are evident in the concentrations of viable populations expressed by ATP, sulphate reducers, iron reducers and the ammonifiers (Table 2). Direct microscopic counts of algae, bacteria and mould did not yield differences between the samples, but their presence is likely playing a role in decomposition of the amendment.

As could be expected in AMD with high concentrations of Cu and Zn, ATP concentrations were low (1.2 ng/ml). Similarly low concentrations of ATP were determined in the coal AMD. This could be the effects of stress on the microbial populations. The high metal concentrations in the waste rock AMD and the altered conditions in the bog which received AMD for 4 month are possible stress factors.

The difference in ATP concentrations between the two samples collected in the uranium AMD, 150 ng/ml and 16 ng/ml is noteworthy. The sample at station I was collected where a pH pocket was detected (pH 4.6 at the bottom of straw layer), whereas sample B was not collected in an area with an elevated pH pocket. The high ATP concentrations and elevated pH noted in the field may well be related.

A discussion of the differences noted in population densities between the samples of the sulphate reducers is plagued with several well-known problems (Holland, Knapp and Shoesmith, 1987). Several types of media for enumeration of sulphate reducing bacteria have been employed. Three of the media, API-RP38 (American Petroleum Institute), Postgate B and Postgate E have many similarities. All media use lactate as the main carbon source, and all media contain the same concentration of yeast extract (a source of vitamins and other micronutrients) and ascorbic acid (reducing agent).

The API medium mainly differs from Postgate B & E media in mineral content and, as well, not contain thioglycollic acid, a

reducing agent, used to ensure a suitable redox potential. Postgate B and E differ mainly in that Postgate E contains agar as a solidifying agent.

The differences in the media provide subtle benefits from one application to another. We have not obtained sufficient data to recommend one medium over others. Overall, the population counts show the presence of sulphate reducers in the microbial ecosystems sampled.

Enumerations of the iron reducing bacteria indicated that population sizes are very low in coal AMD, whereas in most locations where the total iron concentrations are high, the population sizes are high (Table 3). However, no inferences can be made concerning the iron reducing populations' activity until ferric/ferrous concentrations ratios can be directly determined under field conditions in solutions bathing these microbial populations.

The presence of ammonifiers in the samples are indicate that organic nitrogen is converted to ammonia. Concentrations of nitrate were <10 mg/l in most samples. The highest counts ($>10^{5}$/ml) were determined in the coal AMD.

CONCLUSIONS

The elevated pH measured in pockets in the different AMD test areas, along with the reductions in concentrations of Al, Fe and Zn in the coal AMD, suggests that microbial generation of alkali

is brought about by different groups of bacteria. It could be postulated that, in coal AMD, the ammonifiers are of importance, whereas in the uranium and pyrrhotite AMD, the iron reducing bacteria are the dominant populations generating alkali. The relative importance of sulphate reducers and ammonifiers in the different AMD microbial environments can not be delineated at present.

It can be concluded that sulphate and iron reduction can be induced in AMD waters, resulting in alkali generation in the absence of a wetland vegetation and without lime addition. However, before microbial alkali generation can be optimized for the treatment of AMD, all major aspects of the microbial ecology have to be understood. Further field and laboratory investigations will yield the relevant information for the development of the ARUM process - Acid Reduction Using Microbiology.

ACKNOWLEDGMENTS:

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Table 1: Acid mine drainage characteristics in water associated with organic matter.

Table 2: The values of pH and the concentrations of Al, Fe and Zn in water with visible film, compared to water with no amendment and no visible film.

Table 3: Direct microscopic counts and enumerations of alkali generating microbial populations in organic matter samples.

Test	Cu/Zn Tailings	Cu/Zn Waste Rock		Uranium		Coal		Pyrrhotite	
				Stn. I	Stn. D	(5 Years)	(4 Months)	Sample 1	Sample 2
	Straw	Straw	Sawdust	Straw	Straw	Straw	Organics	Straw	Water
Free Mineral Acidity (as CaCO3. ppm)	1.8	4.6	1.0	400	900	71	184	442	96
pH	3.3	4.2	4.3	3.2	2.6	6	3.6	3.25	3
Cu	0.03	7.2	36	1.3	0.1	<0.01	<0.01	<0.01	<0.01
Fe	0.02	3.9	25	1299	290	31	74	1942	1729
Zn	3.3	112	226	3.8	0.6	<0.01	0.2	1.3	0.4
Sulphate (as ppm SO4=)	1320	1650	2910	1440	3930	300	75	7320	6810

Table 1: Kalin, Cairns, and McCready

Parameters		pH		Al (mg/L)		Fe (mg/L)		Zn (mg/L)	
Sample Type		29-May (n=63)	25-Jun (n=14)	29-May (n=12)	25-Jun (n=12)	29-May (n=12)	25-Jun (n=12)	29-May (n=12)	25-Jun (n=12)
Visible Film	MAX	6.7	7.0	0.1	0.1	1.1	9.8	0.2	0.01
	MIN	3.4	5.2	0.01	0.1	0.01	8.6	0.01	0.01
No Visible Film	MAX	4.5	4.0	15.0	4.8	20.0	16.0	4.9	2.4
	MIN	3.2	3.1	0.5	1.6	0.3	0.8	1.3	0.8
No Ammendment	MAX	4.1	3.9	18.0	6.1	58.0	43.0	1.0	1.3
	MIN	3.4	3.1	2.6	4.9	0.1	23.0	0.1	0.3

Table 2: Kalin, Cairns, and McCready

Direct Microscopic Counts (cells/mL)

Test	Cu/Zn Tailing	Cu/Zn Waste Rock		Uranium		(5 y. AMD) Coal		(4 mo. AMD)		Pyrrhotite		
	Straw	Straw	Sawdust	Stn. I Straw	Stn. D Straw	Straw Liquid	Straw Solid	Straw Liquid	Straw Solid	Sample 1 Straw	Sample 2 Straw	Sample 2 Water
ATP (ng/mL)	110	3.7	1.2	150	16	6	16	0.32	3.2	10.3	19	9.3
	*	*	*	*	**	**	**			*	*	*
Algae (10E/mL)	<4	5	<4	<4	4	4	4	<4	<4	5	4	5
Bacteria (10E/mL)	8	7	8	8	7	7	7	6	7	6	6	6
Molds (10E/mL)	5	<4	5	<4	<4	4	<4	<4	4	5	<4	<4
Sulphate Reducing Bacteria (10E/mL)	E 1 F 3	E 3 F >5	E <0 F 1	E 2 F 3	E 2 F 1	3	3	2	2	E 2 F 2	E 2 F 1	E 1 F 1
Iron Reducing Bacteria (10E/mL)	3	>5	4	3	>5	<0	<0	>4	>4	>5	4	>5
Ammonifiers (10E/mL)	3	3	3	4	3	>5	3	1	3	4	3	2

E and F are for Postgate E and F respectively.

* - Mainly diatoms

** - Euglena mutabilis

Table 3: Kalin, Cairns, and McCready

SECTION 3: Dearborn: Microbial Work Presently in Progress

SECTION 3: STAGES OF PROCESS DEVELOPMENT

Based on the work carried out to date and summarized in Section 2 it is possible to define an approach for the microbiological work for the program. We can identify five stages of development, each one requiring optimization.

I Pre-Initiation 1989/90

The selection and possibly treatment of the amendment prior to addition to acid waters. Treatment might include inoculation with bioaugmentation products (eg. cellulose degraders), ozonation, or other types of oxidation (H_2O_2, permanganate) to assist cellulose degradation and/or pre-incubation in diluted site waters to promote microbial adaptation.

II Initiation 1989/90

This stage refers to the development of microbial processes which neutralize highly acidic water (eg.=pH3). In weakly buffered waters or at higher pHs, this stage is essentially absent. The goal of this stage is to raise the pH to pH5 with minimal nucleation and lag period.

III Active Treatment 1990/91

In this stage, incoming acidic seepage is neutralized and an environment favourable for the establishment of cattails is established. At the beginning, continuous cellulose decomposition will be essential for the production of nutrients for alkalinity generating processes. As nitrogen supplies are exhausted, this occurs faster than cellulose decomposition in organic amendments, nitrogen fixation or further supplementation of organic or chemical nitrogen may be required.

IV Initiation of Self-Sustaining System 1991/92

In this stage, toxic metals must have been neutralization and acid neutralization must be stable.

V Maintenance of Self-Sustaining System 1991/92

Inspection, monitoring, and indicator practices must be developed.

ARUM Process: Microbiology Laboratory Milestones and Budget Requirements - Update September 1989

1.0 Milestone #1

Exploration of Process Capabilities and Development of Initiation Stage of Process.

1.1 Execution Period

Pre-April 1989 - December 1989.

1.2 Budget Requirement

$54,000

1.3 Major Completed Accomplishments

1.3.1 Laboratory reactors have been constructed to examine the microbial ecology of alkalinity generation.

1.3.2 Alkalinity reduction was successfully demonstrated in these reactors 8 out of 8 cases.

1.3.3 It was demonstrated that the process is stable to acid shock reactors in which alkalinity generation is well established. Two reactors have recovered from four (4) sequential shocks.

1.3.4 It was demonstrated that cattail litter can stimulate H_2S production and alkalinity production.

1.3.5 It was demonstrated that the following treatments were not successful in initiating alkalinity generation in acid-shocked ecosystems which are inadequately established:

- 200 ppm yeast extract powder
- 200 ppm $(NH_4)\ H_2PO_4$
- ground cattail litter
- 2500 ppm lactic acid
- inoculation from active reactors

1.3.6 A complex solid nutrient was found which rapidly initiated alkalinity generation in an acid-shocked ecosystem.

1.3.7 The following microbial activities were demonstrated to occur in the alkalinity generating ecosystem from Pre-Bog Acid Creek:

- cellulose degradation
- volatile fatty acid production
- sulfate reduction
- ammonification
- iron reduction

1.3.8 In the Pre-Bog Acid Creek ecosystem, evidence was obtained which indicated that iron-reducing bacteria were the major cause of alkalinity generation. Sulfate reduction was sufficient to cause metal precipitation, eg. iron sulfides.

1.3.9 Development of the ARUMATOR concept.

1.4 Accomplishments to be Completed by December 1989

1.4.1 Determine essential microbial activities for process initiation of highly acidic sites (pH =3.5).

1.4.2 Determine a practical nutrient supplement for process initiation in highly acidic sites to complement peat/hay amendments.

1.4.3 Complete the exploration of process capabilities of the Pre-Bog Acid Creek microbial ecosystem (eg. maximum extent of sulfate reduction, further definition of iron reduction capabilities, define chemical vs. biological nature of recovery from acid shock).

1.4.4 Continue to further the development of field sampling/testing techniques (including sampling substrates, anaerobic handling, development of a tiered protocol to assess evolution of alkalinity generating populations in amended sites).

1.4.5 Assess alternate culture techniques for measurement of sulfate reducing bacteria (eg. Starkey's medium).

1.4.6 Establish reactors with Makela ecosystems.

SECTION 4: Boojum: Work in Progress

SECTION 4

BOOJUM WORK IN PROGRESS

Since the issue of the second milestone report, three main aspects have been addressed:

1. Test Cells

The test cells have been completed. An overview of the configuration is given in Schematic 1. In Plate 1, the test cells are depicted.

The water in the seepage test cells has been sampled and analyzed. The results are presented in Table 1, and indicate that no significant change has occurred as the water passes through the system. A slight increase in acidity may be noted, comparing the feed water with 340 mg $CaCO_3$ equiv. with 539 mg/l $CaCO_3$ equiv. Some settling is occurring of the total suspended solids throughout the system. This is expected and is expressed by a difference between Cell 1, with 77 mg/l and Cell 3 with 18 mg/l.

2. Organic Matter Placement

The ongoing field experiments from which the placement techniques will be derived are being harvested. It is

expected that this harvesting will be completed by the end of September. The evaluation methods carried out on sub-samples taken from the field have been successful. The sub-samples, taken from the field and the laboratory reactors show differences. We believe that we now have the experimental tools and measures for determination of the most effective means of placing the amendment.

The data will be summarized by the end of the month and will be discussed in detail at the upcoming meeting at Cooper Cliff on September 29.

Table 1: Water

SAMPLE DATE / SAMPLE VOLUME / ASSAYERS CODE		21-Aug-89	21-Aug-89	21-Aug-89
SAMPLING LOCATION		Cell 3 Inflow	Cell 3 Outflow	System Outflow
Temp.		19.2	19.2	21.2
pH		4.6	4.45	4.47
Cond. (umhos)		3290	3260	3300
Ferric (ppm)		0	0	0
Ferrous (ppm)		250	260	240
ELEMENTS(PPM)	Ag	< 0.0046		< 0.0046
	Al	< 0.0646		< 0.0646
	As	0.0332		0.0352
	B	0.281		0.273
	Ba	0.0167		0.0163
	Be	0.0007		0.0005
	Bi	< 0.0208		< 0.0208
	Ca	447		432
	Cd	< 0.0026		< 0.0026
	Co	0.388		0.376
	Cr	0.0072		0.0074
	Cu	0.0649		0.0729
	Fe	210		200
	Mg	147		141
	Mn	3.73		3.6
	Na	145		142
	Ni	10.7		10.4
	P	0.0987		0.093
	Pb	< 0.0496		< 0.0496
	Sb	< 0.0708		< 0.0708
	Se	< 0.0818		< 0.0818
	Sn	0.0229		0.0252
	Te	< 0.0954		< 0.0954
	Ti	0.01		0.0102
	V	< 0.0041		< 0.0041
	Zn	0.151		0.151
	Chloride	81		85.5
	Acidity	536		539
	Sulphate	3170		3030
	Ammonia	7.6		7.5
	T.S.S.	18		15

Table 1: Water quality for test cells - Makela

SAMPLE DATE / SAMPLE VOLUME / ASSAYERS CODE		21-Aug-89		21-Aug-89	21-Aug-89
SAMPLING LOCATION		Feed Water		Set Pond 1 Inflow	Set Pond 1 Outflow
Temp.			17	16.4	16
pH			6.62	5.5	5.75
Cond. (umhos)			1700	2800	3000
Ferric (ppm)			0	0	0
Ferrous (ppm)			220	260	260
ELEMENTS(PPM)	Ag	<	0.0046	< 0.0046	
	Al	<	0.0646	< 0.0646	
	As		0.0287	0.0315	
	B		0.21	0.251	
	Ba		0.0173	0.0171	
	Be		0.0006	0.0006	
	Bi	<	0.0208	< 0.0208	
	Ca		408	440	
	Cd	<	0.0026	< 0.0026	
	Co		0.176	0.365	
	Cr		0.0066	0.0091	
	Cu		0.015	0.0697	
	Fe		155	252	
	Mg		90	144	
	Mn		1.89	3.44	
	Na		140	144	
	Ni		5.29	9.6	
	P		0.0885	0.0976	
	Pb	<	0.0496	< 0.0496	
	Sb	<	0.0708	< 0.0708	
	Se	<	0.0818	< 0.0818	
	Sn		0.02	0.0277	
	Te	<	0.0954	< 0.0954	
	Ti		0.0261	0.0137	
	V		0.0048	< 0.0041	
	Zn		0.064	0.141	
Chloride			88.5	41	
Acidity			340	460	
Sulphate			3100	3010	
Ammonia			6.7	7.5	
T.S.S.			131	99	

Table 1: Water

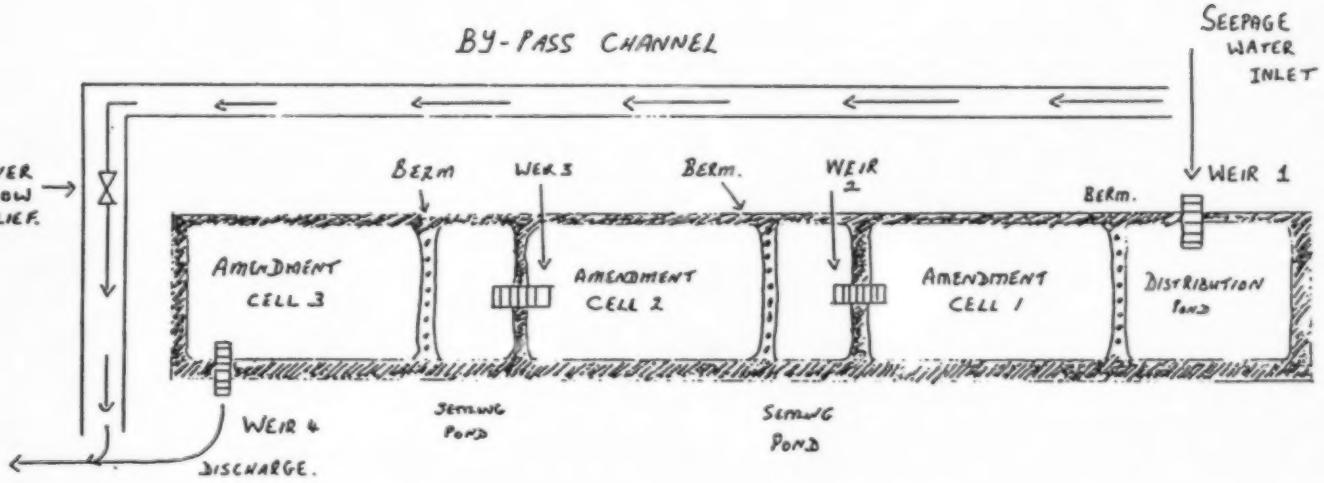
SAMPLE DATE / SAMPLE VOLUME / ASSAYERS CODE		21-Aug-89	21-Aug-89	21-Aug-89	21-Aug-89
SAMPLING LOCATION		Cell 1 Inflow	Cell 1 Outflow	Set Pond 2 Inflow	Set Pond 2 Outflow
Temp.		15.5	16.4	15.1	16.8
pH		5.66	5.52	5.48	5.51
Cond. (umhos)		2700	3000	2450	3100
Ferric (ppm)		0	0	0	0
Ferrous (ppm)		250	250	250	250
ELEMENTS(PPM)	Ag	< 0.0046		< 0.0046	
	Al	< 0.0646		< 0.0646	
	As	0.0384		0.0374	
	B	0.261		0.292	
	Ba	0.017		0.0179	
	Be	0.0008		0.0006	
	Bi	< 0.0208		< 0.0208	
	Ca	432		468	
	Cd	< 0.0026		< 0.0026	
	Co	0.366		0.392	
	Cr	0.0095		0.0093	
	Cu	0.0625		0.0547	
	Fe	233		241	
	Mg	142		153	
	Mn	3.4		3.65	
	Na	141		157	
	Ni	9.61		10.5	
	P	0.0994		0.083	
	Pb	< 0.0496		< 0.0496	
	Sb	< 0.0708		< 0.0708	
	Se	< 0.0818		< 0.0818	
	Sn	0.0248		0.023	
	Te	< 0.0954		< 0.0954	
	Ti	0.0121		0.0114	
	V	0.0047		< 0.0041	
	Zn	0.137		0.147	
Chloride		80.5		80	
Acidity		536		563	
Sulphate		3010		3070	
Ammonia		7.9		6.9	
T.S.S.		77		47	

Table 1: Water

SAMPLE DATE / SAMPLE VOLUME / ASSAYERS CODE		21-Aug-89	21-Aug-89	21-Aug-89	21-Aug-89
SAMPLING LOCATION		Cell 2 Inflow	Cell 2 Outflow	Set Pond 3 Inflow	Set Pond 3 Outflow
Temp.		17.8	19.1	19.8	20.1
pH		5.25	5.19	5.2	5.12
Cond. (umhos)		3050	3350	2980	3350
Ferric (ppm)		0	0	0	0
Ferrous (ppm)		240	220	250	240
ELEMENTS(PPM)	Ag	< 0.0046		< 0.0046	
	Al	< 0.0646		< 0.0646	
	As	0.0328		0.0355	
	B	0.268		0.281	
	Ba	0.018		0.0178	
	Be	0.0008		0.0005	
	Bi	< 0.0208		< 0.0208	
	Ca	476		471	
	Cd	< 0.0026		< 0.0026	
	Co	0.406		0.399	
	Cr	0.0096		0.0079	
	Cu	0.0566		0.061	
	Fe	232		228	
	Mg	156		154	
	Mn	3.71		3.81	
	Na	152		153	
	Ni	11.1		11.1	
	P	0.0992		0.1	
	Pb	< 0.0496		< 0.0496	
	Sb	< 0.0708		< 0.0708	
	Se	< 0.0818		< 0.0818	
	Sn	0.0264		0.0248	
	Te	< 0.0954		< 0.0954	
	Ti	0.0153		0.0124	
	V	< 0.0041		< 0.0041	
	Zn	0.178		0.151	
Chloride		83		80.5	
Acidity		546		575	
Sulphate		3110		3140	
Ammonia		7.5		6.6	
T.S.S.		31		26	

Plate 1:

An overview of the test cells at Makela.



OVERVIEW OF CONSTRUCTED TEST CELLS.